THE CREATINE CONTENT OF THE MUSCLES AND SOME OTHER TISSUES IN FISHES.

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Available data on the creatine content of fish flesh are few in number and imply surprisingly large differences between different species (1, 2). If early reports are neglected, and those only noted in which the analytical method used was based on Folin's, there are on record just twenty-three determinations of muscle creatine within the class Pisces, as strictly defined.[1] The number of species included in the enumeration is fifteen, but most of these are represented by only a single analysis. The record includes figures as high as 835 mg. per 100 gm.[2] (for *Pagrus major*) and as low as 180 mg. (for the "red" muscles of *Auxis tapeinosoma*) (3). Each of these extremes lies far outside the known range for the muscles of mammals. In view of this situation it seemed worth while to seize an opportunity, recently afforded, of examining, for comparative purposes, the tissues of some of the commoner fishes procurable on the coast of British Columbia. The series secured included fifteen species from thirteen genera, three only of which (*Squalus*, *Oncorhynchus*, and *Raia*) appear in earlier lists. Each genus, with two exceptions, was represented by at least two individuals, some by as many as four. Attention was of course directed chiefly to the creatine content of the skeletal muscles; but in several cases the heart or the testis or both were also analyzed. Another creatine-containing organ, the brain, was examined in two instances only, those of the dogfish and the

[1] This excludes two analyses of lamprey muscle.

[2] This includes the creatine equivalent of 70 mg. of "free" creatinine, most of which, it may be assumed, existed in the living muscle as creatine.

skate; no other species yielded enough brain tissue for an accurate determination by the method employed.

An entirely just comparison of different species would require that all be obtained and examined under identical conditions. This requirement it was not possible to fulfil. Some specimens were caught by hand-line, others by set-line, others by seining. Others again were taken from a pen, in which they had been confined for varying lengths of time. Those freshly caught without undue struggle were presumably in a normal state of nutrition. Those which had been maintained in captivity probably all suffered from either partial or complete fasting; while those captured in the open were landed in different degrees of muscular exhaustion. These varying conditions were presumably not without influence upon the creatine content of the muscles. In one respect only could all individuals be treated alike. Each, as brought to shore, was killed promptly before asphyxiation by a blow on the head, and the chemical analysis was started in every case immediately after death.

The method of analysis used was essentially the same as that proposed by Baumann and Hines (4) for mammalian muscle, the modifications introduced being similar to those employed by Harding and Eagles (5) in the determination of creatine in brain. From 4 to 5 gm. of tissue, cut up into small pieces, were weighed by difference into a 6 $\times$ 1 inch test-tube, containing 12.5 cc. of 5 N sulfuric acid. The tube was covered by a watch-glass, and heated for 4 hours in a boiling water bath. The product of hydrolysis was filtered, cooled, and made up with the filter washings to a volume of 25 cc. A 10 cc. portion of this diluted extract was then pipetted into a 50 cc. volumetric flask, and its creatinine content determined by following exactly, from this point, the procedure used by Baumann and Hines. The creatinine solution employed as a standard contained 1 mg. of creatinine per cc., and was made by dissolving 1.602 gm. of creatinine zinc chloride[3] in 1 liter of 0 1 N HCl. With skeletal muscle a suitable amount of this standard was found to be 5 cc. (containing 5 mg. of creatinine), and a suit-

[3] The specimen of creatinine zinc chloride employed contained, according to a Kjeldahl determination, 22.90 per cent of nitrogen (theoretical, 23.19 per cent), and yielded, in comparison with a bichromate standard, the theoretical amount of color.

able dilution for the final colorimetric comparison 250 cc. With heart, testis, or brain (employed in the quantity specified) the standard had to be reduced to 1 mg. (1 cc.) and the final dilution to 100 cc. When, as not infrequently happened, the whole amount of such tissue available was less than 3 to 4 gm., these quantities were further reduced to 0.5 mg. and 50 cc. respectively. All results were translated directly into terms of creatine, the assumption being made that none of the tissues contained more than a negligible proportion of free creatinine.

When the procedure described was applied, by way of control, to the mixed flesh from the hind legs of a rabbit, it indicated in two separate 5 gm. samples a creatine content of 0.564 and 0.567 per cent respectively. A 50 gm. sample of the same flesh, treated according to the unmodified method of Baumann and Hines, gave the value 0.564. These results, while above the average for rabbit muscle, are within the range observed by Folin and Buckman (6), and their agreement shows that the small size of the samples used in the present work is not likely to have been a source of error.

This conclusion is strengthened by the outcome of some parallel analyses made on fish material itself. Thus two separate portions of skeletal muscle from Fish 165 (*Sebastodes ruberrimus*) yielded respectively 0.496 and 0.491 per cent of creatine; two others from Fish 156 (*Leptocottus armatus*) gave 0.561 and 0.558; while two portions of cardiac muscle from Fish 150 (*Ophiodon elongatus*) gave 0.167 and 0.163. The excellent agreement of these duplicates showed that reliance might well be placed upon a single analysis, and in the majority of instances a single analysis only was performed.

The species examined and the individual results obtained are listed in Table I, while Table II shows the position of each species in the systematic classification of fishes, together with an average value, calculated from Table I, for the creatine content of its tail muscles. In both tables the nomenclature adopted is that of Jordan and Evermann (7).

In the mammal different muscles are known to present quite frequently considerable differences of creatine content; so that no comparison of species is entirely fair, unless it is based upon analyses of homologous muscles or muscle groups. It seemed

TABLE I.

Creatine Distribution in Individual Fishes.

Genus and species.	Local name.	Serial No. of fish.	Length of fish.	Description of skeletal muscle analyzed.	Creatine per 100 gm. of: Skeletal muscle.	Heart.	Testis.
			in.		*mg.*	*mg.*	*mg.*
Squalus sucklii.	Dogfish.	152	16	Caudal.	625		
	"	153	15	"	632		
	"	168	32	"	595		
	"	154	36	"	546	63	
		154		Fetal.	460		
	"	155	26			77	137
Raia binoculata.	Skate.	164	36	Caudal.	486	97	169
		164		Lateral.	498		
		164		Red.	240		
	"	167	17	Lateral.	460		
Hydrolagus colliei.	Ratfish.	166	20	Caudal.	545		152
	"	180	23	"	552		
Clupea pallasii.	Herring.	148	7	"	728	214* (148, 149, 151)	
	"	149	7	"	750		
	"	151	8	"	743		
Oncorhynchus kisutch.	Coho salmon.	182	20.5	"	630	189 (182, 181)	
	" "	181	21.5	"	642		
	" "	133	21			163	117
Phanerodon furcatus.	Perch.	147	11	Caudal.	737		
	"	162	10	"	653		
	"	171	9	"	615		
	"	183	12	"	670		

Tæniotoca lateralis.	Blue perch.	184	11	"	604		
	" "	185	10.5	"	622		
Sebastodes maliger.	Rock cod.	145	14	"	531		
" *caurinus.*	" "	146	9	"	527		
" *ruberrimus.*	Red rock cod.	165	23	"	494		105
Hexagrammos superciliosus.	Rock trout.	197	16	"	615		
Ophiodon elongatus.	Ling cod.	138	27	Anterior dorsal.	523	131	133
		138		Midventral.	569		
		138		Caudal.	612		
	" "	143	24	Anterior dorsal.	525	128	144
		143		Midventral.	577		
		143		Caudal.	641		
	" "	131	21	"	575		
	" "	150	46			165	
Scorpænichthys marmoratus.	Sculpin.	161	23	Caudal.	609		138
Leptocottus armatus.	Bullhead.	156	10	"	560		
	"	157	10	"	567		
Platichthys stellatus.	Flatfish.	139	17	"	613		
	"	140	13	"	602		
	"	141	19	"	624		
	"	176	21	"	596		

* For this analysis the ventricles of twelve herrings were combined.

desirable to ascertain at the very beginning how far it might be necessary to apply a similar limitation in the case of fishes. For this purpose three samples of flesh were taken from each of two ling cod (*Ophiodon elongatus*). The first was taken from the dorsal region just behind the head, the second from the abdominal wall about midway between the head and the tail, and the third

TABLE II.

Average Creatine Content of Tail Muscles in Different Species.

Subclass.	Order.	Genus and species.	Average creatine content of tail muscles.	No. of specimens averaged.
			per cent	
Selachii.	Cyclospondyli.	*Squalus sucklii.*	0.600	4
"	Batoidei.	*Raia binoculata.*	0.481	3*
Holocephali.	Chimæroidei.	*Hydrolagus colliei.*	0.549	2
Teleostomi.	Isospondyli.	*Clupea pallasii.*	0.740	3
"	"	*Oncorhynchus kisutch.*	0.636	2
"	Acanthopteri.	*Phanerodon furcatus.*	0.669	4
"	"	*Tæniotoca lateralis.*	0.613	2
"	"	*Sebastodes ruberrimus.*	0.494	1
"	"	" *maliger.*	0.531	1
"	"	" *caurinus.*	0.527	1
"	"	*Hexagrammos superciliosus.*	0.615	1
"	"	*Ophiodon elongatus.*	0.609	3
"	"	*Scorpænichthys marmoratus.*	0.609	1
"	"	*Leptocottus armatus.*	0.564	2
"	"	*Platichthys stellatus.*	0.609	4

* Only one of these specimens was from the tail, the other two being taken from the lateral region.

from the compact mass of muscular tissue posterior to the cloacal aperture. In each instance (see Fish 138 and 143, Table I) there was revealed a progressive and quite conspicuous increase of creatine concentration from before backward, so that much the highest value was found in the powerful propelling muscles of the tail. Accordingly in practically all other cases the sample for

analysis was taken from the caudal region; and, in computing, for Table II, a series of comparable averages, data from other regions were, with one exception, rejected. The exception was made in the case of the skate, in which the laterally disposed muscles of the pectoral fins, upon which in this form propulsion mainly depends, were found to contain quite as much creatine as those of the tail.

A compilation of all recorded data for the creatine content of normal *mammalian* muscles (1) shows only a few isolated observations which surpass the level of 600 mg. per 100 gm. The mixed flesh of the rabbit, which is relatively rich in creatine, contains on the average only 525. The muscles of most other mammals yield less than 500. A glance at Tables I and II shows that the range of creatine concentrations in the flesh of fishes is in general decidedly higher. In seven out of the thirteen genera represented the average concentration (Table II) is close to 0.6 per cent. In three (*Hydrolagus*, *Sebastodes*, and *Leptocottus*) it lies between 0.5 and 0.6; in one (*Phanerodon*) between 0.6 and 0.7. In one only (*Raia*) does it fall below 0.5, while there is one also (*Clupea*) in which it exceeds even 0.7.

Earlier estimates have not always indicated for fish muscle such high levels of creatine content as those now reported. Mellanby (8) found only 0.28 per cent in the flesh of the skate, and 0.35 per cent in that of the cod. Similar low values are given by Cabella (9) for *Phycis brasiliensis* and *Atherinichtys platensis*. On the other hand Okuda[4] (3) reports for the shark and the snapper (*Pagrus major*) the quite remarkably high figures of 0.811 and 0.835 respectively. In better accord with the range now observed are the results of Palladin and Wallenburger (10) for *Lota vulgaris* (0.512 to 0.541 per cent), those of Okuda for *Cyprinus carpio* (0.510), *Katsuwonus pelamis* (0.440 to 0.805), *Oncorhynchus tschawytscha* (0.638), and *Thunnus schlegeli* (0.571), and those of Eggleton and Eggleton (2) for *Cottus* (0.410), *Raia clavata* (0.440), and the dogfish (0.460). It is probably a fair comment upon these rather discordant data that the lowest values are the ones most likely to be in error.

The highest figure in Table II (0.74 per cent) is found in one of

[4] The figures quoted as from Okuda include in every case the creatinine equivalent of a considerable quantity of "free" creatinine.

the Teleostomi (*Clupea*), the lowest (0.48) in a selachian (*Raia*). Among the Holocephali, sometimes grouped with the Selachii as elasmobranchs, we find, in the ratfish, the relatively low value of 0.55. On the other hand the dogfish, another elasmobranch, may have as much as 0.63 per cent of muscle creatine, while certain teleostean genera, like *Sebastodes* and *Leptocottus*, may yield no more than the ratfish. There is therefore no sufficient evidence of general systematic differences between any of the major divisions into which the fishes are grouped.

As a matter of fact there may exist within a single order variations nearly as great as any that may be found between members of different subclasses. There is a notable contrast, for instance, between *Clupea* and *Oncorhynchus* among the Isospondyli or between *Phanerodon* and *Sebastodes* among the Acanthopteri. The species itself, indeed, has no sharply fixed content of muscle creatine. Individual perches (*Phanerodon furcatus*), for example, gave figures all the way from 0.615 per cent to 0.737. In spite of this the range for any species is evidently fairly characteristic, and if the example of *Sebastodes* is typical this statement may be extended to include all species within a single genus.

In many fishes, although the bulk of the muscles is almost white, there are found in certain situations sheets or bundles of muscular tissue of a dark red color. The amount of this tissue available is usually rather small; but in the large skate, Fish 164, it was grouped in conspicuous bundles, easily dissected out, lying ventral to the pectoral fin-rays, and furnishing ample material for a creatine analysis. As indicated in Table I, this red muscle contained just half as much creatine as the pale muscles of the same individual. This confirms similar observations made by Okuda (3) on the red and pale muscles of the bonito (*Katsuwonus pelamis*) and the frigate mackerel (*Auxis tapeinosoma*). It has long been known that the dark meat of rabbits and fowls contains much less creatine than the white.

Another similarity between fish, on the one hand, and mammals or birds on the other, is found in the comparatively low creatine content of developing as compared with adult muscle. This is exemplified by Fish 154, a female dogfish from which were obtained five nearly viable embryos about 7 inches long. The mixed

flesh of these contained only 0.46 per cent of creatine as against 0.55 for the parent fish.

The creatine of living mammalian (or amphibian) muscles, it has been shown, exists for the most part, if not wholly, in a state of combination as "phosphocreatine" or "phosphagen" (11). Upon the removal of the muscle from the body this compound decomposes rapidly into free creatine and phosphoric acid. The phosphoric acid thus liberated constitutes the so called "labile" or "phosphagen phosphorus" of the muscle. In mammalian muscle, therefore, creatine and labile phosphorus occur in equimolecular proportions. If the creatine of fish muscle likewise existed exclusively as phosphocreatine, such muscle should contain not only more creatine than the mammalian, but also more labile phosphorus. Actually it would seem as a rule to contain less. Eggleton and Eggleton (2) report relatively small quantities of phosphagen phosphorus in two out of four fishes examined; while in the muscles of seven different fishes Irving and Wells (12) found no evidence of the presence of any labile phosphorus whatsoever. The precise significance of these findings remains for the present obscure; but evidently it cannot be safely assumed that creatine exists in fish muscle in exactly the same state as in mammalian.

In mammals notable quantities of creatine are known to occur not only in the skeletal muscles but also in the heart, the brain, and the testes. It was to be presumed that in fishes also each of these organs contains creatine, although the only evidence in point is the recent isolation by Steudel and Suzuki (13) of creatinine, as the zinc chloride compound, from the testes of the herring. I have made no attempt at isolation, but the outcome of routine quantitative determinations leaves no reasonable doubt that in fishes as in mammals creatine is a regular constituent of each of the three tissues in question.

In the testes the quantities found (see Table I) are fairly uniform, ranging (in the eight examples secured) between 105 and 169 mg. per 100 gm., with no evident difference between teleost and elasmobranch. The even scantier data available for mammalian testes indicate a probable average in the neighborhood of 200 mg. The fish's testis is apparently less richly supplied with creatine than the mammal's.

For the heart also the nine results obtained with five fish species fall distinctly below the general mammalian level. In thirty-three analyses of cardiac muscle from cats, dogs, rabbits, and sheep Folin and Buckman (6) found creatine percentages between 0.186 and 0.339. In the present series only two figures (for herring and salmon) fall within even the lower part of this range. In the three teleosts represented there seems to be a parallelism between the creatine content of the heart and that of the voluntary muscles. The selachians (or elasmobranchs) occupy, it would appear, a separate position. Both in the dogfish and in the skate the heart has much less creatine than in any of the Teleostomi examined. This, as a matter of fact, is the one point concerning creatine distribution in which there has appeared to be any systematic difference between these two subclasses.

As stated already, the brain was subjected to a creatine analysis in two instances only. The brain of the dogfish, Fish 155, was found to contain 144 mg. of creatine per 100 gm., that of the skate, Fish 164, 116 mg. If these results can be taken as representative, the brains of fishes contain quite as much creatine as those of mammals.

SUMMARY.

The creatine content of the skeletal muscles has been determined in fifteen species of fishes. Considerable differences have been found to exist not only between different species, but even between different individuals of the same species. Each species nevertheless presents a fairly characteristic range of creatine values.

The differences between species do not correspond in any obvious way with zoological subdivisions. In particular there is no systematic difference, with respect to muscle creatine, between the Teleostomi and the elasmobranchs. The latter, however, do show a decidedly lower concentration of creatine in the heart than the former.

In general the skeletal muscles of fishes contain more creatine than those of mammals. Mammals on the other hand show a higher concentration of creatine in the heart and in the testes. The two analyses made of selachian brain tissue indicate a content of creatine equal to that of mammalian brains.

In fishes, as in mammals and birds, red muscles contain less creatine than pale, and fetal muscle less than the adult.

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